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THE DIETARY DEFICIENCY OF CEREAL FOODS
WITH REFERENCE TO THEIR CONTENT
IN "ANTINEURITIC VITAMINE"

By CARL VOEGTLIN, G. C. LAKE, AND C. N. MYERS

THE GROWTH-PROMOTING PROPERTIES
OF FOODS DERIVED FROM
CORN AND WHEAT

By CARL VOEGTLIN AND C. N. MYERS

PHOSPHORUS AS AN INDICATOR OF THE
"VITAMINE" CONTENT OF CORN
AND WHEAT PRODUCTS

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The Dietary Deficiency of Cereal Foods with Reference to Their Content in "Antineuritic Vitamine."¹

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Introduction.—Recent experimental work has called attention to the importance of certain so-called accessory foods or vitamins in their relation to proper nutrition. The work of Stepp, Hopkins, Funk, Osborne and Mendel, McCollum and Davis, and others, has demonstrated that a diet is not complete and does not permit of adequate nutrition unless it contains sufficient amounts of two accessory substances. One of these substances is essential in the prevention and cure of beriberi in man or polyneuritis of fowls, and has been termed the "antineuritic vitamine" or the water soluble B. The other substance occurs in certain foods rich in fat (butter, etc.), and has been designated as fat soluble A by McCollum and his coworkers.

This paper deals with the occurrence of the antineuritic vitamine in foods derived from wheat or corn. Researches of a similar nature have been made by other investigators, and the fact has been thoroughly established that all natural cereals contain an abundance of antineuritic vitamine, an amount considerably in excess of that needed when these cereals form the exclusive diet of animals.

Valuable as these investigations are in regard to the feeding of animals and the purely scientific aspects of nutrition, the previous investigators have not sufficiently taken into consideration the fact that cereals in the form of the entire grain are only rarely consumed by man. The bulk of the cereal component of the diet of our people is represented by wheat or corn bread. Wheat and corn bread are largely prepared from flour or corn meal which represents only part of the wheat or corn kernel. The commonly used "patent" flour, for instance, contains only about 70 per cent of the wheat from which it is milled. The question, therefore, arises as to whether or not bread made from "white" flour or "highly milled" corn meal includes all the essential food elements contained in the intact grain.

The present paper reports a continuation of work done by others with respect to the antineuritic vitamine of corn and wheat products

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used in nutrition. In a succeeding paper certain other dietary attributes of these foods will be considered in their relation to growth.

Previous work.—Experimental and clinical work on the etiology of beriberi has demonstrated the fact that polished rice may give rise to beriberi in man and to polyneuritis in fowls when used to the more or less complete exclusion of other foods. It has, furthermore, been shown that substitution of unpolished rice for polished rice prevents the occurrence of beriberi and polyneuritis. Moreover, it has been found that the rice polishings contained the antineuritic vitamine, the substance which is responsible for the prevention and cure of beriberi. Thus unquestionable proof was furnished that the “highly milled” (polished or white) rice has been deprived of an essential dietary element resident in the peripheral layers of the intact grain.

Because of these results with rice, the attention of investigators interested in this field was soon attracted to wheat bread. Thus Holst (1907), in experimental studies relating to ship beriberi and scurvy, carried out on pigeons a few experiments with rye and wheat bread, baked with yeast or baking powder. The pigeons on wheat bread developed symptoms and nerve degeneration characteristic of polyneuritis. The addition of yeast to the wheat flour seemed to delay the onset of the symptoms, but did not prevent a gradual loss of body weight (30 per cent). The pigeons fed on rye bread or whole wheat bread, however, lived for four months without showing any abnormalities. Holst does not mention the kind of wheat flour used in his experiments beyond stating that it was “the flour ordinarily used by Norwegian sailors in tropical waters.”

Simpson and Edie (1911) describe a few additional experiments on the feeding of “whole wheat” bread and “white” bread to pigeons. On the “whole wheat” bread the birds continued active and well, and two pigeons paired and successfully hatched the two eggs. The pigeons fed “white” bread died after an average of 29 days with an average loss of weight of 26 per cent. Typical paralytic symptoms were observed in these animals and marked degenerative changes were found in their peripheral nerves. Several of these birds were treated when extremely weak with either yeast or beans. Great improvement in the condition of the birds followed this treatment.

The reports of Little (1912 and 1914) concerning his five years’ experience with epidemics of beriberi in Newfoundland are very important, as they show that in this locality beriberi was due to a diet limited to “white” bread made from highly milled flour, molasses, a small quantity of fish, and tea. This author furthermore presents evidence to the effect that the substitution of “whole wheat” bread for the “white” bread was followed by a disappearance of the dis-

case, in spite of the fact that the other dietary and hygienic conditions were apparently left unchanged.

At Little's suggestion, Ohler (1914) carried out a number of experiments with chickens, in order to furnish experimental proof for the contention that a more or less exclusive diet of "white" bread was the chief cause of the occurrence of beriberi in Newfoundland. Fourteen chickens fed on "white" bread, made from "highly milled" flour with or without the addition of yeast, died within 28 to 40 days. Before death the birds exhibited symptoms of polyneuritis, and histological examination of the peripheral nerves revealed considerable degeneration. Five fowls fed on "whole-wheat" bread and two on whole wheat lived in perfect health for 75 days, when the experiment was discontinued.

Similar experiments were carried out by Wellmann and Bass (1913) and by Voegtlin, Sullivan, and Myers (1916).

Nature of the present experiments.—Pigeons and chickens were used in these experiments for the reason that they are better adapted for feeding experiments with cereals than other animals. Only full-grown birds were used. The symptoms resulting from a deficiency of the diet in antineuritic vitamine are easily recognized in fowls, and curative experiments yield striking results. The observations made on birds were confirmed in experiments on dogs, hogs, and a large number of white mice (these results will be published elsewhere). As a rule, the birds were given as much of the various corn and wheat foods as they cared to eat. In a few experiments, by mistake they did not receive sufficient food, a fact which caused them to lose in body weight (experiments Nos. 18, 20, and 25). The appearance of polyneuritic symptoms was regarded as indicating that the particular food in question was deficient in antineuritic vitamine. The polyneuritic symptoms were always preceded by a gradual loss in body weight. The peripheral nerves of a large number of the birds were examined histologically at the end of the period of observation, and always confirmed the conclusions drawn from clinical observations. Birds exhibiting polyneuritic symptoms during life always showed marked myelin degeneration of their peripheral nerves.

The corn and wheat products were obtained in the open market and came from various modern roller mills, old-fashioned buhr mills and various bakeries.

As a rule, the total phosphorus pentoxide content of the dried products was determined, as it had been shown by other investigators that the phosphorus and vitamine contents of rice of various degrees of milling run roughly parallel.

In order definitely to prove that the symptoms observed in the animals kept on deficient foods were due to a deficient supply of

antineuritic vitamine, some of the pigeons were treated after the onset of the disease with various vitamine preparations (Funk's vitamine fraction) obtained from brewer's yeast, ox liver, or rice polishings. Further evidence was obtained from protective experiments in which there was added to the deficient food a small quantity of a vitamine preparation. This material was prepared according to the method described by Seidell (1914). The so-called activated Lloyd's reagent consists of fuller's earth (Lloyd's reagent), which has been treated with autolyzed yeast filtrate. It contains an average of 2.3 per cent of total nitrogen, which is derived from the basic substances of autolyzed yeast filtrate.

As it was confirmed in the course of the investigation that white bread was deficient in antineuritic vitamine, a few experiments were made to determine the effect of adding an extract of "whole-wheat" bread to the diet of "white" bread. This extract was prepared as follows: Two hundred and eighty grams of "whole-wheat" bread were extracted twice at room temperature with one liter of 50 per cent alcohol containing 25 cubic centimeters of concentrated hydrochloric acid. The filtered extract was concentrated in vacuo to a small volume, so that 1 cubic centimeter of the extract corresponded to 1 gram of fresh "whole-wheat" bread. The extract was free from fat and contained 11.5 per cent of solids.

The animals were kept in wire cages, tap water being supplied daily and grit from time to time.

The bread was fed in the form in which it was received from the dealer. Water was added to the flour, which was made into cakes. The cakes were dried at 45° C. and broken up into pieces of suitable size.

Throughout this paper the words "highly milled" are meant to indicate that the product was milled in a manner to eliminate most of the superficial layers and the germ of the grain. "White" bread is the ordinary wheat bread made from "highly milled" flour. "Patent" flour is the word applied by the milling industry to a flour which is of very fine texture and almost white in appearance. It contains practically no bran or germ. By "old-fashioned" Graham flour is meant a wheat flour which is obtained by grinding wheat in buhrstone mills with the omission of bolting. "Whole-wheat" flour is a designation of a flour which contains the whole-wheat berry, with the exception of part of the bran. "First-clear" flour resembles "patent" flour but contains a small amount of the peripheral portions of the grain. Similar designations have been used for the corn foods. The following tables and charts will illustrate the main results obtained in this investigation.

EXPERIMENT 1.—"Old-fashioned" Graham flour milled in the presence of one of authors (V) in an old-fashioned buhr mill in Spartanburg, S. C.

[Appearance: Typical Graham flour, containing considerable bran and germ. P_2O_5 content: 0.866 per cent.]

(Beginning with the twenty-fifth day of the experiment, a similar Graham flour was substituted for the above product.)

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
		<i>Days.</i>		
308	No symptoms.....	118	+ 2	Sciatic normal.
309	do.....	118	+ 9	Do.
312	do.....	123	+21	Sciatic not examined.
313	do.....	96	+12	Do.
311	do.....	113	+13	Do.

Remarks.—On the 25th day of the experiment the food was changed to another old-fashioned Graham flour ($P_2O_5=0.82$ per cent), obtained from another buhrstone mill in Spartanburg, S. C. This mill had been in operation for at least 50 years. Slight changes were made in its power plant, substituting turbines for the "water wheels." The process of milling, however, was still the same as at the time of the opening of the mill.

Both of these flours contained sufficient antineuritic vitamine to prevent polyneuritic symptoms and loss of body weight. Their P_2O_5 content is almost as high as that of whole wheat.

EXPERIMENT 2.—"Whole wheat flour" milled in St. Louis, Mo.

[Appearance: Grayish white. P_2O_5 content: 0.61 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
		<i>Days.</i>		
332	No symptoms.....	90	+21	Sciatic normal.
333	do.....	90	-28	Do.
336	do.....	91	-33	Do.
337	do.....	103	-22	Do.
335	do.....	96	-36	Do.

Remarks.—Pigeon 337 died apparently from accidental causes, as the necropsy revealed a piece of copper wire, which had perforated the craw and prevented the animal from eating. The other pigeons appeared in perfect health until a few days previous to end of period of observation. No definite paralytic symptoms were observed in any of these birds and the histological examination of the sciatic nerves also yielded negative results.

This flour contained sufficient antineuritic vitamine to prevent polyneuritic symptoms and fatty degeneration of the peripheral nerves. Its P_2O_5 content is somewhat lower than that of whole wheat.

EXPERIMENT 3.—*Wheat flour obtained from old-fashioned buhr mill, Roebuck, S. C.*[Appearance: White, with yellowish tinge; contains particles of bran. P_2O_5 content: 0.265 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
315	No definite symptoms.....	Died after 109 days.....	-36	No treatment.
317	Twenty-second day.....	Died after 27 days.....	-15	Do.
318	No definite symptoms.....	Killed after 158 days.....	-28	Do.
319	No symptoms.....	do.....	+ 4	Do.

Remarks.—Sciatic nerves of pigeons 315 and 319 did not reveal any degenerative changes and were to all appearances normal. Sciatic nerve of pigeon 318 revealed a slight degeneration.

This flour represents a fairly “highly milled” product. It was bolted through a fine silk cloth, which eliminated a considerable part of the germ and bran. In physical appearance this flour is not as refined as “patent” flour. It obviously contains more antineuritic vitamine than “patent” flour but not as much as “whole-wheat” flour. The P_2O_5 content is also low.

EXPERIMENT 3a.—*Wheat flour obtained from old-fashioned buhr mill, Spartanburg, S. C.*[Appearance: White with yellowish tinge; contains small particles of bran. P_2O_5 content: 0.27 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
338	Remained well.....	Killed after 118 days.....	-13	Not treated.
339	No symptoms.....	Died after 102 days.....	-12	Do.
340	Remained well.....	Killed after 118 days.....	- 6	Do.
341	do.....	do.....	- 5	Do.
343	Thirtieth day, doubtful.....	Died after 38 days.....	-34	Do.

Remarks.—Sciatic nerve of pigeon 339 showed slight degeneration. There were never noticed any definite symptoms of polyneuritis in this bird. Sciatic nerve of pigeons 338 and 340 did not exhibit any degeneration; that of pigeon 341 revealed slight degeneration, although the bird seemed to be in perfect health when it was killed.

EXPERIMENT 4.—“*Patent wheat flour*” obtained from a Maryland roller mill.[Appearance: Pure white; no bran can be detected. P_2O_5 content: 0.25 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
326	Twenty-second day.....	Died after 43 days.....	-22	Relieved symptoms on thirtieth day by an extract of rice polishings.
327	Fifteenth day.....	Died after 67 days.....	-44	Relieved symptoms on thirtieth and fifty-seventh days by yeast vitamine.
328	do.....	Died after 26 days.....	-21	Not treated.
329	Tenth day.....	Died after 16 days.....	-31	Do.
330	do.....	Died after 12 days.....	- 7	Do.
331	Twenty-second day.....	Died after 46 days.....	-20	Relieved symptoms on twenty-eighth day by an extract of rice polishings.

Remarks.—Pigeon 326 received, on thirtieth day of experiment, 0.5 cubic centimeter of Funk's vitamine fraction from an extract of rice polishings. Bird was greatly improved as a result of this treatment, but gradually developed a relapse several days later. Pigeon 327 received, on thirtieth and fifty-seventh days of experiment, 0.5 cubic centimeter autolyzed yeast vitamine, prepared by means of Lloyd's reagent (dose equivalent to 40 cubic centimeters autolyzed yeast filtrate).

The pigeons of this series all developed polyneuritis within three weeks. The flour was "highly milled" and had a low phosphorus content. The administration of an active preparation of antineuritic vitamine promptly relieved the symptoms.

EXPERIMENT 5.—"*Patent*" wheat flour obtained from a roller mill in Virginia.

[Appearance: Pure white; no particles of bran or germ. P_2O_5 content: 0.20 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
344	Fifteenth day.....	Died after 29 days.....	-37	Not treated.
345	Twenty-second day.....	Died after 30 days.....	-34	Do.
346	Tenth day.....	Died after 26 days.....	-43	Do.
347	Fifteenth day.....	Died after 39 days.....	-37	Do.
348	Tenth day.....	Died after 36 days.....	-14	Temporary improvement after administration of "vitamine."
349	Fifteenth day.....	Died after 19 days.....	-28	Not treated.

Remarks.—All of the pigeons of this lot developed typical polyneuritis between the tenth and twenty-second days after feeding was begun, and died with marked loss in body weight. Pigeon 348 was given one dose of 0.3 cubic centimeter of a fluid antineuritic preparation, obtained from autolyzed brewers' yeast, which caused a temporary improvement in the symptoms.

EXPERIMENT 6.—"*Patent flour*" milled in a Michigan roller mill.

[Appearance: Pure white; contains no bran. P_2O_5 content: 0.206 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
320	Tenth day.....	Died after 38 days.....	-27	Not treated.
321	Fifteenth day.....	do.....	-13	Do.
322	Eighteenth day.....	Died after 34 days.....	-43	Symptoms relieved on the eighteenth day by a preparation made from rice polishings.
323	Fifteenth day.....	Died after 18 days.....	-38	Not treated.
324	do.....	Died after 40 days.....	-30	Do.
325	do.....	Died after 83 days.....	-43	Symptoms relieved on twentieth and thirty-third days by a preparation made from yeast.

Remarks.—Pigeon 322 received 0.5 cubic centimeter of a dilute extract of rice polishings (vitamine fraction), which caused complete disappearance of severe symptoms of paralysis. Pigeon 325 was given, on the twentieth day, 0.5 cubic centimeter of an extract of brewers' yeast (vitamine fraction); severe paralytic symptoms disappeared within 24 hours. On the thirty-third day another attack of polyneuritis was completely relieved by a dose of 1 cubic centimeter autolyzed yeast vitamine, prepared

by means of Lloyd's reagent (dose equivalent to 40 cubic centimeters autolyzed yeast filtrate). The pigeon remained free from any symptoms after this treatment for about 25 days.

EXPERIMENT 7.—“*Straight patent flour*” milled from winter wheat at a roller mill in South Carolina.

[Appearance: White; contains a few particles of bran. P_2O_5 content: 0.216 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
351	Twentieth day.....	Died after 36 days.....	-27	Not treated.
352	do.....	Died after 38 days.....	-39	Do.
353	Twenty-sixth day.....	Killed after 118 days.....	-34	Relieved symptoms on twenty-sixth day by a preparation made from ox liver; on thirty-fourth day with yeast preparation. Diet changed on Fortieth day.
354	Twentieth day.....	Died after 52 days.....	-28	Relieved symptoms on twenty-sixth day by a preparation made from ox liver.
350	Twenty-sixth day.....	Died after 57 days.....	-48	Not treated.

¹ Fortieth Day.

Remarks.—Diet of pigeon 353 was changed, on fortieth day of experiment, to a wheat flour obtained from an old-fashioned buhrmill in South Carolina. This change caused the pigeon to regain some of the lost weight (original weight 249 grams, weight at period of dietary change 165 grams; weight at end of experiment, 43 days later, 188 grams). Pigeon was killed after 118 days, being free from symptoms of polyneuritis. Sciatic nerve did not reveal any degenerated fibers.

This experiment, in conjunction with those preceding, shows that a “highly milled” flour with low phosphorus content leads to polyneuritis within about three weeks. The symptoms are due to a deficiency of this food in antineuritic vitamine, as demonstrated by the curative effect of a small quantity of an antineuritic extract from yeast or ox liver.

EXPERIMENT 8.—“*Patent wheat flour*” obtained from a Minnesota roller mill through the open market.

[Appearance: Pure white; no bran can be detected. P_2O_5 content: 0.25 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
		<i>Days.</i>		
1	Twentieth day.....	25	-39	Died of polyneuritis; sciatic degenerated.
2	Eighteenth day.....	37	-40	Do.
3	Thirty-first day.....	38	-33	Severe paralysis relieved with antineuritic material.
4	Twenty-second day.....	38	-35	Do.
5	Twelfth day.....	17	-22	Died of polyneuritis; sciatic degenerated.
6	Twenty-eighth day.....	36	-24	Do.

Remarks.—On this flour the birds developed polyneuritis in about the same time as on highly milled rice. Marked degeneration of the sciatic was found on histological examination. The P_2O_5 content of the flour was low (less than one-third that of "whole wheat").

EXPERIMENT 9.—"White-wheat bread" obtained from a bakery in Washington, D. C.

[Appearance: Good; no bran. P_2O_5 content: 0.26 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
		<i>Days.</i>		
37	Forty-second day.....	45	-36	Polyneuritis cured by extract of "whole-wheat bread."
38	Forty-fifth day.....	80	-42	Not treated; died of polyneuritis; sciatic degenerated.
39	Fifty-fourth day.....	64	-23	Severe polyneuritis cured by extract of "whole-wheat bread."
40	Eighteenth day.....	40	-38	Do.
41	Thirty-first day.....	35	-41	Not treated; died of polyneuritis sciatic degenerated.
42	Seventeenth day.....	40	-39	Do.

Remarks.—The bread used in this experiment was made from a mixture of "patent" and "first clear" flour, yeast, sodium chloride, compound lard, molasses, and evaporated milk. The exact formulas could not be obtained. This bread had a low phosphorus content and led to polyneuritic symptoms in the pigeons of this series in about three to five weeks. It is seen that the incubation period is somewhat longer (three to five weeks) than in birds kept on "patent" flour (2 to 3 weeks). This incubation period is also longer than that observed when polished rice forms the exclusive diet of pigeons. Three of the pigeons were given an extract of the "whole-wheat" bread used in experiment 15. Chart 3 illustrates the gradual loss in body weight on a diet of white bread and the slow recovery after treatment with the extract of "whole-wheat" bread was begun. The addition of yeast and evaporated milk to the flour, in the process of bread making, did not seem to furnish sufficient antineuritic vitamine to prevent polyneuritis.

EXPERIMENT 10.—White-wheat bread, ordinary baker's bread, purchased on the open market in Washington, D. C., and Spartanburg, S. C.

[Appearance: White; contained no bran. P_2O_5 content: 0.26 and 0.19 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Died after—	Change in body weight in per cent of original weight.
		<i>Days.</i>	
116	Thirty-second day.....	32	Not determined.
120	Twentieth day.....	21	Do.
300	Twenty-fifth day.....	42	-15.
304	Twenty-first day.....	29	-29.
305	Twenty-fourth day.....	43	-4.
307	Twenty-fifth day.....	46	-18.
401	Fourteenth day.....	15	Not determined.
402	Fortieth day.....	40	Do.

Remarks.—From these experiments it would seem that the incubation period of polyneuritis is somewhat prolonged (about 14 days) as a result of the addition of yeast and evaporated milk to the highly milled flour in the process of bread making. Both yeast and milk are known to contain antineuritic vitamine.

EXPERIMENT 11.—*High grade "patent" wheat flour + Lloyd's reagent (inactive). This flour was obtained from a Minnesota roller mill and 1 per cent of Lloyd's reagent incorporated with it.*

[Appearance: White; no coarse particles. P_2O_5 content: 0.25 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
		<i>Days.</i>		
13	Thirty-first day.....	38	-43	Experiment discontinued after 38 days.
14	Thirty-eighth day.....	38	-27	Do.
15	Twenty-fifth day.....	38	-40	Died of polyneuritis; sciatic nerve degenerated.
16	Thirty-first day.....	38	-38	Experiment discontinued after 38 days.
17	do.....	38	-32	Do.
18	do.....	54	-48	On fifty-fourth day pigeon was dying; killed with chloroform; sciatic degenerated.

Remarks.—This experiment shows that "patent" flour, with the addition of Lloyd's reagent (fuller's earth) which has not been activated by autolyzed yeast, does not prevent the appearance of polyneuritis in pigeons. These results are in marked contrast with experiments illustrated by chart 5.

CHART 1.—Experiment 12. Weight curve of pigeons on “highly milled” (patent) wheat flour. All of the pigeons of this group developed polyneuritis within 21 days and lost considerable body weight. Four of the birds died after having been on this diet 17, 26, 36, and 37 days, respectively. The two remaining birds received an extract of “whole wheat” flour and were cured of their paralytic symptoms, but did not recover their lost body weight during the next 40 days.

CHART 2 (Continuation of Chart 1).—Experiment 12. Recovery of body weight of pigeons on diet of "highly milled" (patent) wheat flour, as a result of the addition to the flour of 1 per cent activated Lloyd's reagent. The previous administration of an extract of "whole wheat" flour had cured the pigeons of the paralytic symptoms, but had not led to a recovery of body weight. The amount of extract was probably not sufficient for this purpose.

CHART 3.—Experiment 13. Weight curve of pigeons on diet of “white” bread. Symptoms of polyneuritis appeared a little later than on a diet of “highly milled” flour (see Chart 1). Two of the animals died of polyneuritis; the others were treated with an extract of “whole wheat” bread and were cured of their symptoms. Later on (Chart 4) the surviving pigeons received daily 50 mg. of activated Lloyd’s reagent (containing antineuritic vitamin) by stomach.

CHART 4 (Continuation of Chart 3).—Recovery of pigeons on diet of "white bread" as a result of the daily administration of 50 mg. of activated Lloyd's reagent.

CHART 5.—Experiment 14. Weight curve of pigeons on diet of "highly milled" (patent) wheat flour + 0.5 per cent activated Lloyd's reagent, showing prevention of loss of body weight in pigeons on this mixture. In another experiment, the same amount of *inactive* Lloyd's reagent was added to the patent flour. The pigeons developed polyneuritis within three weeks and lost about 30 per cent of body weight, thus showing that fullers' earth per se can not prevent polyneuritis.

CHART 6.—Experiment 15. Weight curve of pigeons on diet of "whole wheat" bread, showing that "whole wheat" bread contains sufficient antineuritic vitamine to prevent a loss in body weight of pigeons fed exclusively on this diet. The birds of this series did not develop any symptoms of polyneuritis.

CHART 7.—Experiment 16. Weight of pigeons on diet of "whole wheat" flour. This chart shows that the pigeons fed on "whole wheat" flour alone do not lose weight. This food is adequate as the sole source of antineuritic vitamine. No symptoms of polyneuritis were observed in any of these birds.

EXPERIMENT 17.—*Whole corn.*[P₂O₅ content: 0.76 per cent.]

No. of chicken.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.
6	No symptoms.....	<i>Days.</i> 222	—14
10	do.....	428	— 5
11	do.....	188	— 2

Remarks.—The animals of this group were healthy, full-grown chickens. They were kept in individual small cages throughout the period of observation. The lack of cage space may account for the trivial loss in body weight, as it is very difficult to keep fowls in perfect condition in such narrow confinement for so long a time.

EXPERIMENT 18.—“*Old process water-ground corn meal*” obtained from an old-fashioned buhr mill in Georgia.[Appearance: Contains large particles of bran and germ. P₂O₅ content: 0.659 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
66	No symptoms.....	<i>Days.</i> 150	—30	Not treated.
67	do.....	73	—36	Do.

Remarks.—Pigeons 66 and 67 remained perfectly well, but lost considerable weight. This loss of weight was due to the underfeeding of these birds. They received only 10 grams corn meal daily, instead of 15 to 20 grams. In spite of the loss of weight, these animals did not exhibit the slightest signs of polyneuritis during the long period of observation.

EXPERIMENT 19.—*Corn grits (hominy)* obtained from an old-fashioned buhrstone mill in South Carolina.[Appearance: Practically all the bran and germ mixed with the finer endosperm. P₂O₅ content: 0.668 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
98	No symptoms.....	<i>Days.</i> 152	—11	Not treated.
99	No symptoms; escaped.....	98	(¹)	Do.
96	No symptoms.....	77	+9	Do.

¹ Not determined.

EXPERIMENT 19a.—Same as used in experiment 19, except that the larger particles of bran were removed by passing through a coarsely meshed sieve.

[P₂O₅ content: 0.59 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
		<i>Days.</i>		
102	No symptoms.....	152	— 8	Not treated.
103	do.....	152	— 6	Do.
100	do.....	152	—10	Do.

EXPERIMENT 20.—Corn meal used at Milledgeville State Hospital, Milledgeville, Ga.

[Appearance: Contains considerable bran and germ. P₂O₅ content: 0.677 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
		<i>Days.</i>		
72	No symptoms.....	52	—38	Not treated.
73	do.....	84	—15	Do.

Remarks.—Both pigeons were underfed, receiving only 10 grams of cornmeal daily. This fact accounts for the loss of body weight.

Pigeon 73 also received old process corn meal, obtained from the United States Penitentiary, Atlanta, Ga. (0.77 per cent P₂O₅). This bird remained well and apparently in perfect health for a period of 84 days when the experiment was discontinued. No symptoms of polyneuritis were exhibited.

EXPERIMENT 21.—Corn grits obtained from State institutions in Alabama and Georgia.

[Appearance: Highly milled. P₂O₅ content: 0.169 and 0.201 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
61	Twenty-second day.....	41 days.....	¹ —22	Beginning with twenty-second day, daily dose 0.2 c. c. yeast extract.
62	Twentieth day.....	45 days.....	(²)	Beginning with twentieth day, daily dose 0.2 c. c. yeast extract.
64	Eighteenth day.....	Died after 34 days.....	—48	Not treated.
411	do.....	Died after 31 days.....	—33	Do.

¹ Twenty-second day of experiment.

² Not determined.

Remarks.—Pigeons 61 and 62 received grits which was used in the State hospital in Alabama (P₂O₅ content=0.169 per cent). Pigeon 64 received grits used in a hospital for the insane in Georgia (P₂O₅ content=0.201 per cent). All of these products contained very little bran and germ and were products of roller mills. Pigeons 61 and 62 responded very well to the treatment with yeast extract (disappearance of polyneuritic symptoms) and were in excellent condition at the end of the period of observation.

EXPERIMENT 22.—*“Highly milled” corn grits obtained from a roller mill in North Carolina.*[Appearance: Contains a trace of bran and germ. P_2O_5 content: 0.21 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
91	Thirty-first day.....	Died after 72 days.....	Not determined...	Symptoms relieved on sixty-fourth day by yeast extract.
92	Twenty-third day.....	Died after 92 days.....	do.....	Improved on sixty-seventh day by alcoholic extract of yeast.
93	Thirty-first day.....	Died after 91 days.....	do.....	Improved on seventy-third day by yeast extract.
94	Forty-seventh day.....	Died after 95 days.....	do.....	Marked improvement on sixty-fifth day by 0.2 gm. alcoholic yeast extract.
97	Thirty-first day.....	Died after 64 days.....	do.....	Not treated.

EXPERIMENT 23.—*Highly milled corn grits and wheat bran. The grits were obtained from a roller mill in North Carolina and the bran from an old-process (stone) mill in South Carolina. The grits were the same as used in Experiment 22.*[P_2O_5 content of corn grits: 0.21 per cent. P_2O_5 content of wheat bran: 2.48 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Diet.
87	No symptoms.....	149 days.....	0	35 gm. corn grits+10 gm. wheat bran.
85	do.....	do.....	+ 3	
86	do.....	do.....	— 9	
88	Sixty-seventh day.....	Died after 68 days.....	(¹)	40 gm. corn grits+5 gm. wheat bran.
89	One hundred and forty-ninth day.....	226 days.....	+ 8	
90	One hundred and fiftieth day.....	Died after 138 days.....	—31	

¹ Not determined.

Remarks.—Pigeons 85, 86, and 87 remained in perfect condition throughout the period of observation. Pigeons 88, 89, and 90 developed polyneuritic symptoms after a considerable length of time and two of the birds died of the disease. Pigeon 89, however, recovered from the paralytic attack, regained its lost body weight and appeared perfectly well at the end of the period of observation (226 days).

EXPERIMENT 24.—*Highly milled corn grits with addition of corn bran. The grits were the same as used in experiment 22, and the bran was obtained from old-process corn meal by passing the latter through a wide-meshed sieve.*[P_2O_5 content of corn grits: 0.21 per cent. P_2O_5 content of corn bran: 0.787 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
81	Seventy-third day.....	Died after 113 days.....	Not determined...	Symptoms relieved on seventy-third day by 10 c. c. of dilute yeast extract.
101	do.....	Died after 115 days.....	do.....	Do.

Remarks.—Both pigeons of this lot received a mixture of 15 grams of corn grits and 5 grams of corn bran. The addition of corn bran caused a very considerable delay in the onset of polyneuritis (about 40 days). This furthermore seems to indicate that corn bran is probably not as rich in antineuritic substance as either wheat bran or possibly corn germ. On examination of the corn bran it was noticed that very little of the so-called aleuron layer adhered to the cellulose layer of the bran.

Both pigeons recovered temporarily from their paralytic symptoms as a result of a single dose of an antineuritic yeast preparation. The birds again recovered their lost appetite and remained free from symptoms for 38 days (pigeon 81) and 41 days (pigeon 101), respectively.

In order to discover whether a larger addition of corn bran would offer complete protection from polyneuritis, pigeon 74 was fed on a mixture of 10 grams highly milled corn grits (0.20 per cent P_2O_5) and 10 grams corn bran (0.78 per cent P_2O_5). The corn grits used were the same as fed to pigeon 411 (experiment 21) and had caused symptoms of polyneuritis on the eighteenth day after feeding was begun. Pigeon 74 remained well on this diet for two months, when the experiment was discontinued.

EXPERIMENT 25.—*Corn germ obtained from a roller mill in Indiana.*

[P_2O_5 content: 2.81 per cent.]

No. of pigeon.	First appearance of polyneuritis.	Period of observation.	Change in body weight in per cent of original weight.	Notes.
70	No symptoms.....	Days 85	—17	Not treated.
71	do.....	92	—20	Do.

Remarks.—These pigeons were underfed (see p. 649), receiving only 10 grams of food daily. They never exhibited the slightest symptoms of polyneuritis and were lively throughout the period of observation.

Conclusions.—(1) The results obtained in this investigation clearly show that for pigeons an exclusive diet of whole wheat or corn furnishes an adequate supply of antineuritic vitamine.

(2) The antineuritic vitamine seems to reside in the peripheral layers and the germ of these seeds, whereas the endosperm is relatively poor in this substance.

(3) If wheat and corn foods containing only a small percentage of the peripheral layers and germ of the seed are fed to pigeons and chickens exclusive of other food, polyneuritic symptoms appear on an average of three weeks after the beginning of the feeding period. The appearance of polyneuritis is preceded by a gradual loss in body weight. The birds can be relieved of their paralysis in a striking way by the oral or subcutaneous administration of a highly concentrated preparation of antineuritic vitamine derived from "whole-wheat" bread, yeast, ox liver, rice polishings, or beans.

(4) The addition of yeast (in amounts used by bakers) in the preparation of bread from highly milled flour does not prevent the appearance of polyneuritis in birds fed on this food, but prolongs slightly the period of incubation.

(5) The addition to "highly milled" flour, or bread made from "highly milled" flour, of a small amount of antineuritic vitamine preparation will correct this particular dietary deficiency, and will prevent the appearance of polyneuritis and the loss of body weight.

(6) The total phosphorus content of corn and wheat foods is a fairly satisfactory index of the amount of antineuritic vitamine contained in these foods. In a general way, it can be said that a high total phosphorus content is an indication that the particular corn or wheat product is relatively rich in antineuritic vitamine

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THE GROWTH-PROMOTING PROPERTIES OF FOODS DERIVED FROM CORN AND WHEAT.¹

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The authors in a preceding paper have shown that the whole wheat or corn grain contains an abundance of antineuritic vitamine, whereas the "highly milled" products derived from these cereals are deficient in this respect. This conclusion was drawn from experiments on adult animals. As growing animals require the presence of all dietary constituents to a greater extent than do adults, it seemed desirable to continue the investigation along this line. Recent studies on growth have furnished the necessary information to answer the question as to what constitutes a diet complete enough to insure growth. It is now generally held that a physiologically sufficient diet must contain an adequate caloric value derived from protein of proper composition, carbohydrate, and fat. In addition it must contain a sufficient amount of antineuritic and fat-soluble vitamins and of necessary inorganic salts.

The dietary deficiencies of the whole wheat and corn kernel in the diet of growing animals have already been investigated. Thus Hart and McCollum (1914), working with young albino rats and hogs, have shown that normal growth is obtained when the wheat kernel is supplemented by the addition of inorganic salts, fat-soluble vitamine, and casein. Subnormal growth was observed with rations consisting of wheat plus casein and salts; wheat plus casein and butter fat; wheat plus salts and butter fat.

McCollum, Simmonds, and Pitz (1916) have made similar observations with corn. In this case also the grain requires an improvement in its protein moiety, its salt content, and an added supply of fat-soluble vitamine. Of these three additions the correction of the deficiency in certain inorganic salts seemed to be of the greatest importance, inasmuch as this correction in itself furnished a ration on which rats did grow fairly well for several months.

Hart, Halpin, and Steenbock (1917) report experiments with pullets weighing 2 to 3 pounds on corn and wheat rations. These investigators found that corn and wheat meal do not support growth in this species of animals; fortifying the cereals with an appropriate

¹ Reprint from the Public Health Reports, vol. 33, No. 22, May 31, 1918, pp. 843-868.

inorganic salt mixture, protein of proper composition, and a small amount of fat-soluble vitamine (2 per cent butter fat) leads to normal growth. These authors also call attention to the possibility of intoxication as a result of excessive wheat feeding, which they attribute to the presence of some toxic substance in the wheat kernel.

The purpose of the present investigation was to answer the question as to whether the corn and wheat products used in human nutrition exhibit similar dietary deficiencies as those of the whole grains. The bulk of the corn and wheat foods of the American dietary are derived from the wheat and corn kernel by means of a process of milling (roller mills) which is known to eliminate most of the germ and superficial layers of the grain. It, therefore, seemed to us a question of practical importance to determine whether the milling process improves, or causes a decrease in, the dietary value of the milled product. Moreover, it was desirable to decide whether or not the food additions made to flour (yeast, salt, milk) in the preparation of bread improve the nutritive value of this food.

Experimental.

The experiments were carried out on squabs, young albino mice, and a few hogs. Most of the work was done with wheat, and only a few incomplete experiments were made with foods obtained from corn.

We are not aware of any previous records where squabs have been used for studies on growth. For this reason the following details are given, as they may be of interest to workers in this field. The growth period of pigeons is extremely short, as will be seen from the records. Almost maximum body weight is reached, on an adequate diet, within 40 days after the squabs are hatched. The feathers develop gradually, and by the time full growth is reached the body is completely covered. The birds begin to fly at the age of about 40 days. This is usually followed by a slight loss of body weight, which is probably due to the strenuous muscular work performed in flying. It should be pointed out that squabs can not feed themselves during the first two weeks of life. During this period the parents feed the young by regurgitating food which has been softened in their crops. The function of the crop consists in the preparation of the cereal food for gastric digestion. It is very doubtful whether active digestion takes place within the crop, and it is more likely that this organ simply softens the food by means of water in order to facilitate its disintegration by the stomach. In the absence of the crop, gastric digestion of cereals would be a rather slow process, even in the case of the bird's stomach with its powerful muscular wall and the gravel which takes the place of millstones.

In order to obtain a sufficient number of squabs for experiments on growth, about 70 to 100 healthy pigeons were kept in a well-ventilated room containing numerous cages (wire screened), the doors of which were left open. Usually the birds built their nests in these cages and after laying the eggs the parents were caught, confined to the cage, and put on the diet to be tested for its growth-promoting properties. When it happened that eggs were laid outside of the cages, the pigeons were allowed to hatch and the young squabs were then transferred, with the nest and the parent birds, to the nearest cage. We found that it was impossible to move the eggs, as the birds refused to sit after the eggs had been handled. The sitting period of pigeons is about 17 days. The male bird sits from about 9 a. m. to 5 p. m.; the female from 5 p. m. to 9 a. m.

The stock pigeons were fed for nearly two years, while this work was in progress, on an exclusive diet of corn and wheat, crushed oyster shells, and river sand. Fresh running water was supplied. On this diet the birds bred very well throughout the year, with the exception of the moulting season.

The average egg weighed about 16 grams; the contents, minus the shell, weighed about 15 grams, and the weight of the squab immediately after hatching was about 13 grams. It was found that squabs which were below the average body weight and those that had difficulty in hatching, were of low vitality. The squabs were weighed as soon after hatching as possible and every three days thereafter during the morning hours (9 a. m.). The growth curve during the first 10 days is almost a straight line, slight deviations being due to variations of crop content. The birds which died as a result of a diet of inadequate composition were necropsied and the sciatic nerves examined for the presence of myelin degeneration.

The great advantage of using squabs for growth experiments is due to the possibility of immediately starting the newborn animal on a ration which is to be investigated. It is obvious that this can not be done when mammals are used.

The experiments with albino mice were carried out on young animals weighing approximately 6 to 8 grams and obtained from a healthy stock kept in the laboratory. Recent work has sufficiently demonstrated that mice are as well suited for growth experiments as albino rats. The animals were placed in wide glass jars with a wire screened top. Sawdust was used as bedding. Under these conditions the mice could be kept in excellent health on an adequate diet for nearly a year. The animals were weighed every three or four days.¹

The hogs used were young animals, either purchased on the open market or raised in the laboratory. They were kept in stalls with a concrete floor. The bedding was wheat straw, some of which was eaten by the animals. Tap water was supplied. The experiments on hogs are somewhat complicated, as the straw and tap water may perhaps be considered as a source of inorganic salts and fat-soluble vitamine. However, this factor remained approximately constant in each experiment.

Food used.—When the whole wheat was fed to mice it was usually crushed in an ordinary kitchen mill. The wheat flour, either alone or with other foods, was made into cakes by means of water. The wet cakes were dried at 45° C. and broken up into small pieces. The white flour used was bought under the name of "patent" flour and came from one of the largest roller mills of the West. The wheat "middlings" were obtained from a roller mill in Washington, D. C. The "whole wheat" bread used was purchased in Washington, D. C., and the bakery volunteered the following information in regard to the food materials used in the preparation of this bread: Standard loaves were made from crushed whole wheat, with the addition of canned eggs, some salt, olive oil, molasses, compound lard, wheat bran, and pressed yeast. The accurate proportions of the various constituents could not be obtained. The "white" bread was made from "highly milled" wheat flour, with the addition of sodium chloride, compound lard, yeast, and evaporated milk. For 900 standard loaves of this bread, 588 pounds of flour, 47 pounds of evaporated milk, and 8½ pounds of pressed yeast were used.

In the experiments where the "white" bread was supplemented by other foods, the bread was first dried at 40 to 50° C., crushed in a mill, and mixed with the other food in the desired proportions. The casein was a purified preparation made in this laboratory. The crushed oyster shells, fed to the pigeons, consisted largely of calcium carbonate, with traces of organic matter. When the rations contained chemically pure calcium carbonate in place of the oyster shells, exactly the same results were obtained so far as the growth of squabs was concerned. The grit was

¹ The normal growth curve of mice was taken from the article by Mitchell, J., *Biol. Chem.*, 1916, vol. 26, p. 24.

well-washed river sand, consisting mainly of silicates. The so-called "activated" Lloyd's reagent was used as a source of antineuritic vitamine.¹

In some experiments the "inactive" Lloyd's reagent was included in the rations. Fuller's earth, which had not been in contact with the yeast filtrate, was used in this case. The highest proportion of activated Lloyd's reagent contained in any of the rations was 3 per cent. In most cases 0.6 per cent or 1.5 per cent were used with equal success. When the activated Lloyd's reagent forms 0.6 or 1.5 per cent of the ration, the total nitrogen derived from this source represents only 15 to 37 milligrams.

It should be pointed out that squabs do not well tolerate the addition of considerable quantities of fat to the diet. In some experiments, where 5 per cent of lard was incorporated in the ration, the birds showed diarrhea and poor growth, followed by decline and death.

Summary.

It seems superfluous to enter into a detailed discussion of the results obtained in this investigation, as the accompanying charts illustrate the results in a comprehensive manner. For this reason only the main points will be referred to in this summary.

1. The "highly milled" products are, without exception, inferior in dietary value, as regards growth, to foods prepared from the whole grain. It is rather surprising that such delicate organs as the gastrointestinal tract of young mice can tolerate a diet containing a large amount of bran. This fact, however, does not necessarily mean that it is advantageous to include the bran in foods intended for human nutrition. On the contrary, the experiences with "war bread" would rather indicate that persons with delicate digestion are subject to temporary digestive disturbances as a result of a change from "white" bread to bread containing a considerable percentage of bran ("war bread"). On the other hand, from the standpoint of dietary completeness, a bread including all of the grain, with the exception of the superficial cellulose layer, is undoubtedly superior to the so-called white bread, made from "highly milled" flour, and would not possess the above-mentioned objectionable features.

2. The "white" bread used in these experiments was not adequate for maintaining normal growth, in spite of the fact that it was prepared with some evaporated milk and yeast. The most significant defect of "white" flour is the deficiency in antineuritic and fat-soluble vitamine; it is also deficient in adequate protein and inorganic salts.

3. A wheat flour, containing a considerable part of the germ and superficial layers of the grain, supports growth of mice and pigeons especially well when supplemented with inorganic salts. The same is true of "whole wheat" bread.

¹ It was prepared from autolyzed brewer's yeast by treatment with hydrochloric acid and filtration. This yeast filtrate was then treated with a special grade of fuller's earth, which removed a considerable part of the active vitamine from the yeast filtrate. The dried preparation was free of protein and gave negative tests for tryptophan, cystin, and tyrosin. No lysin could be isolated but the activated Lloyd's reagent contained a substance which in its reactions resembled histidine. The total nitrogen content of the dried reagent was about 2.5 per cent, and this consisted largely of adenine and other basic substances derived from yeast filtrate.

4. "Highly milled" corn grits, forming the exclusive food of young hogs, leads to failure of growth in these animals, whereas the whole corn kernel, supplemented by inorganic salts, promotes growth.

5. Newborn squabs are suitable animals for growth experiments.

6. No evidence of a toxic action of a whole wheat diet was obtained in the experiments on squabs which were fed on whole wheat meal, supplemented by a suitable salt mixture.

7. In the light of our present knowledge, it would appear that bread made from "whole wheat" flour, or old-fashioned corn meal, should be used in preference to "white" bread and "highly milled" corn foods, whenever the diet is restricted to these cereal foods to the more or less complete exclusion of other foods possessing greater dietary values.

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CHART 1.—Shows satisfactory growth of mice when “whole wheat” bread is supplemented with 5 per cent of salt mixture. Evidently the whole wheat bread used in this experiment was slightly deficient in inorganic salts, as seen from chart 2, where the bread was fed without the addition of the salt mixture. The composition of the salt mixture used in this investigation was as follows: NaCl , 0.50 gm.; K_2HPO_4 , 1.21 gm.; $\text{CaH}_4(\text{PO}_4)$, H_2O , 0.256 gm.; Calcium lactate, 2.944 gm.; Ferric citrate, 0.100 gm.

CHART 2.—Illustrates the growth of mice on “whole wheat” bread. The retardation of growth is mainly due to the deficiency of this diet in inorganic salts. (See chart 1.)

CHART 3.—Growth of mice. Shows beneficial effect of the addition of salt mixture to "undermilled" flour (compare with chart 4). Addition of 5 per cent butterfat to the diet (as indicated by arrows) increased rate of growth of mouse 114, but not of mice 110 and 112. From charts 3 and 4 it seems that this "undermilled" flour is deficient in certain inorganic salts and possibly fat soluble vitamins.

CHART 4.—Illustrates the subnormal growth of mice on a diet of "under milled" flour. This flour was obtained from a roller mill and was bought as "second clear." It contained 0.12 per cent P_2O_5 . From the phosphorous content of this flour it would appear that this product is the grade of flour intermediate between a "first and second clear."

CHART 5.—Shows insignificant growth of mice when "white" flour is supplemented with a salt mixture. The orange juice was added to the drinking water with the idea of preventing scorbutic symptoms. The "white" flour was bought under the name of "patent" flour and contained 0.25 per cent of P_2O_5 . The sciatic nerves of mouse 102, 96, and 97 showed marked myelin degeneration. These animals probably died of polyneuritis.

CHART 6.—Illustrates early failure of growth, followed by death of mice on a diet of "white" flour plus salt mixture. The sciatic nerves of these mice showed marked myelin degeneration. The results of this experiment are therefore identical with the one which is illustrated by chart 5.

CHART 7.—Growth of mice. Illustrates beneficial effect of the addition of antineuritic vitamine in the form of activated Lloyd's reagent to a mixture of "white" flour and inorganic salts. Compare with charts 5 and 6. Bread No. 24 is not a complete diet, probably deficient in fat-soluble vitamine and certain essential amino acids.

CHART 8.—Practically no growth (except in mouse 149) was obtained on a diet of "white" bread and salt mixture. The addition of 5 per cent butter fat (as indicated by arrows) caused considerable growth in mouse 150. Mice 145 and 149 showed somewhat better growth as a result of the butter-fat addition, but both animals died later on in the experiment. The sciatic nerve of one animal of this group and which was examined after death showed considerable myelin degeneration. (Indication that this diet is deficient in antineuritic substance.)

CHART 9.—Shows beneficial effect on growth of mice as a result of the addition of casein and lard to bread No. 27 (see chart 8). All of the animals died, however, with the exception of mouse 171. The "white" bread is evidently deficient in antineuritic vitamine and amino acids, essential for normal growth. Bread No. 2 is also deficient in antineuritic vitamine (compare with chart 11). Bread No. 2 is same as bread 27, except that the former is supplemented by casein and lard.

CHART 10.—Illustrates deficiency of bread No. 1 in antineuritic vitamine. Compare with chart 12. All the mice of this series died after periods ranging from 16 to 93 days.

CHART 11.—Bread No. 7, which is deficient in fat-soluble vitamine, leads to fairly good growth in mice. Mice 196 and 201 showed normal growth for a considerable length of time (120 days) and reached nearly maximum weight. The other mice of this series stopped growing after having been on this diet for 40 days.

CHART 12.—Illustrates the fairly good growth of mice on bread No. 8. Completion of growth was not obtained with all the mice of this series. Mice 204 and 206 did not grow well after having reached about 12 gm. of body weight.

CHART 13.—Growth of mice.

CHART 14.—Shows retardation of growth of mice on bread No. 10, deficient in inorganic salts.

CHART 15.—Shows satisfactory growth of squabs on a diet limited to the corn and wheat kernel, supplemented by the addition of calcium carbonate (oyster shells). The oyster shells were crushed and fed to the parents ad libitum. Squab B22 reached normal body weight in 20 days, but died suddenly at the age of 75 days. The cause of death is unknown. The other two squabs of this series showed normal growth and development and lived for 150 days, when the experiment was discontinued. The appearance of the birds at this time was normal in every respect.

CHART 16.—Shows failure of growth of squabs on a diet of whole wheat alone. The birds evidently suffered from privation of mineral salts. The skeleton was poorly developed, calcification being very deficient. When the wheat kernel was supplemented by the addition of calcium phosphate practically normal growth was obtained. Sciatic nerve of Squab B11, B5, and B6 shows no myelin degeneration.

CHART 17.—Shows delayed growth and failure of growth of squabs on "whole wheat bread." This food is deficient in calcium salts. Compare with chart 18. Sciatic nerve of squab A15 did not reveal any myelin degeneration.

CHART 18.—Illustrates normal growth and development of squabs on a diet of "whole wheat" bread and calcium phosphate. The parents of these two squabs raised two other pairs of squabs while being fed on this diet. This proves that growth, reproduction, and maintenance of normal nutrition are possible on a simple diet as "whole wheat" bread supplemented by calcium salts.

CHART 19.—Growth of squabs. Shows beneficial effect of addition of "middlings" and calcium phosphate to "highly milled" flour. The "middlings" fed with the mixture for the first 25 days of the experiment had the appearance of wheat bran and contained 1.88 per cent P_2O_5 . The mixture of "white" flour and "middlings" contained 1.21 per cent P_2O_5 . Compare this chart with chart 23.

CHART 20.—Illustrates failure of growth, followed by death of squabs, on a diet of "white" bread and calcium phosphate (A33, A37, and A38). When the "white" bread is further supplemented by casein and a preparation containing antineuritic vitamine, normal growth and development results. Squabs B70, 72, 811, 833 reached nearly maximum normal weight at the age of about 30 days. Sciatic nerves of A33, A37, and A38 exhibit myelin degeneration.

CHART 21.—Shows satisfactory growth of squabs when the "white" bread is supplemented by protein of proper composition, inorganic salts, and antineuritic vitamin. In this experiment the amount of the latter food accessory is twice as large as in the experiments illustrated by chart 20.

CHART 22.—Growth of squabs. The diet used in this experiment must be considered as physiologically complete. The "white" bread evidently contains sufficient fat-soluble vitamine (derived from evaporated milk) to render this ration adequate for growth.

CHART 23.—Shows good growth of a squab on a diet of “white bread,” which was supplemented by inorganic salts and antineuritic vitamine. Evidently the “white bread” used in this investigation was prepared with sufficient evaporated milk to correct the deficiency of the highly milled flour in fat-soluble vitamine and protein of proper composition.

CHART 24.—Shows subnormal growth of squabs on a diet which is deficient in inorganic salts and antineuritic vitamins. The inactive Lloyd's reagent which was added to the diet had not been treated with autolyzed yeast and therefore did not contain the antineuritic substance. Both birds developed polyneuritic symptoms shortly before death.

CHART 25.—Shows subnormal growth of squabs, followed by polyneuritis and death, when the "white" bread is supplemented by casein and a salt mixture (L2 and L1). The addition of "inactive" Lloyd's reagent, which has not been treated with autolyzed yeast, does not alter the result (B55 and B52).

CHART 26.—Shows that squabs grow normally when the "white" flour is supplemented by inorganic salts, protein of proper composition, fat soluble vitamin, and antineuritic vitamin.

CHART 27.—Illustrates subnormal growth of squabs on a diet which is deficient in protein of proper composition. Compare with charts 28 and 29.

CHART 28.—Growth of squabs on diets deficient in fat soluble vitamin (B90 and B900) or fat soluble vitamin and protein of proper composition (B91 and B911).

CHART 29: WEIGHT CURVE OF PIG IV^B.

CHART 29.—Illustrates the failure of growth of young hogs on a diet of "highly milled" corn grits. This food was obtained from a roller mill and represents the endosperm of the corn kernel. The animal was born of a hog which had been raised in the laboratory on a diet of cracked corn, wheat straw, and tap water. On the corn grits the animal did not gain more than a few pounds during nine months, in spite of the addition of a supplementary salt mixture and antineuritic vitamine. The animal finally died. The necropsy revealed the following abnormalities: Emphysema of lungs, chronic gastritis, small injected areas in small intestine, chronic passive congestion of liver, congestion of spleen, no scorbutic changes. Sciatic shows marked myelin degeneration. Another pig of the same litter showed a similar growth curve on a diet of corn grits. Here also correction of the salt content and antineuritic vitamine of the diet did not prevent death. Necropsy findings were the same as in Fig IVB.

CHART 30:—WEIGHT CURVE OF PIG II ON DIET OF CORN OIL CAKE, FOLLOWED BY WHOLE CORN.

CHART 30.—Illustrates failure of growth of a hog fed on corn oil cake. This product is obtained commercially on a large scale by pressing out the oil from the corn embryo. As soon as this diet was changed to whole corn a rapid increase in the rate of growth followed. This animal reached nearly full size and was in excellent condition at the end of the experiment. Compare this chart with chart 29, where "highly milled" corn grits was fed.

PHOSPHOROUS AS AN INDICATOR OF THE "VITAMINE" CONTENT OF CORN AND WHEAT PRODUCTS.¹

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Previous work by the writers has demonstrated that the vitamins are not evenly distributed throughout the corn and wheat kernel. Thus it was shown that the starchy part of these cereals is very deficient in vitamins, whereas the other portions of the grain (bran and germ)² contain a considerable amount of these substances. These results were obtained from experiments on fowls by feeding the particular cereal product in question and noting the time necessary for the appearance of polyneuritic symptoms. When the symptoms appeared after an average of two to three weeks on a given diet, the diet was considered as containing only an insignificant amount of anti-neuritic vitamin. In case the symptoms appeared after three to four weeks the product was regarded as being deficient, but not entirely lacking in this substance. Finally, if no symptoms appeared within 60 days or longer, the diet was considered adequate in this respect.

Information concerning the fat soluble vitamin was furnished by growth experiments on mice and squabs. In this case it was determined whether or not it was necessary to supplement a particular corn or wheat product with fat-soluble vitamin, in the form of butter fat, in order to produce growth in these animals. When the product did not lead to practically normal growth, in spite of the fact that the other dietary deficiencies of the food with the exception of the fat-soluble vitamin were corrected, the product was considered as being deficient in this substance.

These biological procedures for the approximate estimation of the vitamin content obviously have the great disadvantage of being time consuming. On the other hand, chemical methods for the direct quantitative determination of vitamins are not available. Under these circumstances the search for more convenient means, which would permit at least a rough estimate of these food accessories in cereal foods, is urgently needed.

So far as the fat-soluble vitamin is concerned, an estimation of the fat content of the cereal product might yield the necessary in-

¹ Reprint from the Public Health Reports, vol. 33, No. 23, June 7, 1918, pp. 911-917.

² The terms bran and germ are applied throughout this paper to mill products of a varying degree of purity. Thus the germ is not the pure embryo of the grain, but is always contaminated with some bran. The bran always contains a small amount of the starchy part of the cereal.

formation, inasmuch as it has been found that the germ seems to contain most of the fat as well as the fat-soluble vitamine of the grain. The following figures very clearly show the differences in the fat content of the most important corn and wheat products:

CORN. ¹		WHEAT. ²	
	Fat in per cent.		Fat in per cent.
Corn (whole).....	3. 62	Wheat (whole).....	2. 74
Corn grits.....	. 48	Flour, patent.....	1. 45
Corn meal.....	1. 41	Flour, low grade.....	3. 86
Germ.....	23. 79	Germ.....	15. 61
Bran.....	6. 71	Bran.....	5. 03

There seems to exist a similar relationship between the fat content and the antineuritic substance of wheat and corn foods. In practice this theoretical deduction may very well prove of value.

However, the present investigation was confined exclusively to the relation between phosphorus and vitamine content, for the reason that previous work with rice milled to various degrees had demonstrated the intimate relation between phosphorus and antineuritic vitamine. Thus, Fraser and Stanton (1909) ³ on the basis of a wide experience with beri-beri in the East went even so far as to reject as unsafe any rice with a phosphoric oxide content of less than 0.4 per cent when this food formed the principal article of the diet.

The object of the present communication is to correlate the phosphorus content of corn and wheat foods with their content in antineuritic and fat-soluble vitamine. Special emphasis is placed on the distribution of phosphorus in the various products obtained from modern corn and flour mills.

Experimental.

The phosphoric oxide determinations were made on samples dried at 110° C. to constant weight. Five to ten grams of the dried sample are ashed by the Neumann method. Considerable patience is required in the early stages of the process to prevent excessive foaming. It has been found advantageous to allow the sample to remain in contact with concentrated sulphuric acid for about 24 hours before the ashing with nitric acid takes place. The ashed material is neutralized with ammonia until it is alkaline to litmus. After cooling, enough water is added to bring up the volume to about 250 cubic centimeters and the solution made just acid with nitric acid (litmus). One cubic centimeter of 10 per cent nitric acid and 25 cubic centimeters ammonium nitrate ⁴ are then added. The flask containing the solution is then immersed in a water bath, kept at 65° to 68° C. After the contents of the flasks have reached this temperature, a sufficient amount of molybdate solution ⁵ acidified by

¹ Winton, Burnet and Bornmann, U. S. Dept. Agr. Bull. No. 215 (1915).

² Richardson, Cl. U. S. Dept. Agr. Bull. No. 4 (1884).

³ Fraser and Stanton. 1909. Studies from the Institute of Medical Research, Federated Malay States. The etiology of beri-beri.

⁴ Prepared as follows: 3,750 gm. of ammonium nitrate, C. P., are dissolved in 2,500 cc. of hot distilled water; after cooling to room temperature this solution is diluted with distilled water to 5,000 cc. and filtered.

⁵ This molybdate solution is prepared as follows: 100 gm. of molybdic acid are dissolved in 144 cc. of ammonium hydroxide (sp. gr. 0.90) and 271 cc. of water. This solution is poured slowly and with constant stirring into a mixture of 489 cc. of nitric acid (sp. gr. 1.42) and 1,149 cc. of water contained in a large porcelain dish. This mixture is kept in a warm place for several days, or until a portion heated to 40° C deposits no yellow sediment, and preserved in glass-stoppered bottles.

mixing 5 cubic centimeters of concentrated HNO_3 (sp. gr. 1.42) with 100 cubic centimeters of molybdate solution, is added with constant shaking.

The flasks are then heated for 15 minutes in the water bath. The yellow ammonium phosphomolybdate precipitate is now at once filtered off through a Gooch crucible with asbestos filter. The filtrate is always tested for complete precipitation by adding a fresh amount of ammonium nitrate and molybdate solution and reheating in the water bath. The flask and the precipitate are well washed with a 1 per cent ammonium nitrate solution until neutral to litmus, and finally with a little distilled water. The precipitate is now returned to the flask with the addition of about 200 cubic centimeters of distilled water. To this mixture N/2 sodium hydroxide, measured from a burette, is added until the yellow precipitate is completely dissolved. There should be 5 to 10 cubic centimeters of alkali in excess. One cubic centimeter of neutral phenolphthalein solution is added as indicator and the solution immediately titrated back with N/2 sulphuric acid until the pink color just disappears.

The difference between the sulphuric acid and the sodium hydroxide represents the amount of N/2 sodium hydroxide used to neutralize the insoluble precipitate $(\text{NH}_4)_3\text{PO}_4 \cdot 12\text{MoO}_3$.

One cubic centimeter of N/2 NaOH is equivalent to 1.5440 milligrams P_2O_5 .

In regard to the phosphoric oxide determinations, it should be pointed out that the procedure just described will yield reliable results, as shown by the striking agreement of duplicate analyses. However, the results obtained by this method can not be compared with results obtained by other methods, as different analytical procedures will yield slightly different results.

The following tables give the results of the phosphorus determinations, as well as an estimate of both the antineuritic and fat soluble vitamins of these cereal products. The data referring to the vitamin content are taken from two previous papers by the authors.

In order to illustrate the distribution of the phosphorus of the original grain in the course of roller milling, Table 4 is offered. For a better understanding of this table a brief outline ¹ of the process of milling is added.

The wheat as received by the mill is first put through a cleaning machine, which is known as the "milling separator." This consists of a series of metallic sieves. The perforations of the first two sieves are just large enough for a kernel of wheat to pass through, and therefore, oats, straw, and other impurities larger than wheat are separated from the wheat. The lowest sieve of the series has perforations considerably smaller than a kernel of wheat, which permit the smaller mustard seeds and other impurities to pass through.

From the milling separator the wheat is passed through the "wheat scourer," which consists of an upright perforated cylinder, in the center of which, revolving about the shaft, are large beaters. The wheat falls down in the center, is struck by the beaters, and is thrown against the outer case, and after revolving a number of times against the casing is passed through the bottom of the cylinder. While it is falling through the cylinder, a strong air current, passing upward, carries off the dust from the scourings.

The wheat (61)² now passes to the first set of corrugated rolls. After passing through these rolls it (62) goes into one end of a long reel, covered with coarse bolting cloth. The middlings (63) pass through the cloth and what remains of the wheat goes to the second reduction rolls, which are similar to the first.

This process is repeated five times, and after the middlings are taken out after the fifth reduction, all that is left of the wheat is the bran (79).

Before the middlings can be reduced to flour, the fine bran and dust must be taken out of them, or, in other words, they must be purified. Before purification can be

¹ We are indebted for this description and the samples referred to in Table 4 to the manager of one of the largest flour mills in this country.

² The figures in parentheses in this and the following paragraphs correspond with those of the diagram and represent the serial numbers of the samples used for the phosphorus determinations.

accomplished thoroughly the middlings must be divided into various grades with respect to size. This is accomplished by running the middlings into one end of a long reel which is covered with bolting cloth of various degrees of fineness. On the head of the reel is a very fine cloth through which the flour passes; the next section is covered with a coarser cloth through which the first grade of middlings passes; the cloth of the next section is still coarser, and so on up to the end of the reel. What is too coarse to go through the last section of the reel passes over the end and is called the coarsest middlings (68).

After the middlings are graded they pass on to the "middlings purifier." This is a long narrow sieve with a strong current of air passing upward through the cloth. The middlings travel from one end of the sieve very gradually to the other end. The air current carries off the fine dust into a dust collector, and the fine bran, being lighter than the middlings, is suspended by the air current from the cloth, while the middlings go through the cloth. The bran (69) is carried by the current of air to the tail of the machine, and is thus separated from the middlings (70).

This process is the same for the various grades of middlings. After the purification the middlings are reduced similarly to the whole wheat, except that instead of corrugated rolls smooth rolls are used. The coarsest grade of middlings is ground, then passed on to a reel, covered with a very fine cloth, through which the flour (74) passes and goes to the flour bin. Particles of the middlings that are too coarse to pass through the cloth go over the end of the reel to another set of rolls and are ground again. This process is repeated for all grades of middlings until all have been reduced to flour. The "clears" and lower grades of flour are made from the fine bran and dust that are taken out of the middlings during the process of purification. This is done by grinding between smooth rolls the fine bran and dust. The bran will not break up, but flattens out. The product is passed on to a reel covered with fine cloth, through which the flour passes. This flour contains very fine particles of bran which it is impossible to separate from it, and therefore this flour can not be run into the highest grades of flour. It is sold as either "first clear" (75), "second clear" (76), or "red dog" (77), according to the amount of bran it contains.

Conclusions.

The phosphoric anhydride determination of wheat and corn products yields fairly satisfactory information as to the content of these products in accessory foods. A low phosphoric anhydride content indicates that the product is poor in vitamins.

TABLE 1.—*Corn products—Content in P_2O_5 and vitamins.*

Sample No.	Nature of product.	Source.	Degree of milling.	P_2O_5 in per cent.	Estimated vitamin content (from feeding experiments).	
					Fat-soluble vitamin.	Antineuritic vitamin.
1	Corn, whole.....	Connecticut.....	Not milled.....	0.77	Relatively high.	High.
2	do.....	California.....	do.....	.84	do.....	Do.
3	do.....	Indiana.....	do.....	.73	do.....	Do.
4	do.....	Tennessee.....	do.....	.69	do.....	Do.
5	do.....	Alabama.....	do.....	.57	do.....	Do.
6	do.....	Georgia.....	do.....	.61	do.....	Do.
25	Corn meal.....	Georgia (b u h r mill).	Undermilled; considerable bran and germ.	.77	do.....	Do.
27	do.....	South Carolina.....	do.....	.79	do.....	Do.
83	do.....	Tennessee (buhr mill).	do.....	.67	do.....	Do.
32	do.....	Georgia (b u h r mill).	do.....	.66	do.....	Do.
31	do.....	South Carolina (buhr mill).	do.....	.67	do.....	Do.
36	do.....	do.....	do.....	.63	do.....	Do.
33	Corn grits.....	do.....	do.....	.67	do.....	Do.
30	Hominy.....	do.....	do.....	.64	do.....	Do.

TABLE 1.—*Corn products—Content in P_2O_5 and vitamins—Continued.*

Sample No.	Nature of product.	Source.	Degree of milling.	P_2O_5 in per cent.	Estimated vitamine content (from feeding experiments).	
					Fat-soluble vitamine.	Antineuritic vitamine.
38	Corn bran.....	South Carolina (buhr mill).	Undermilled; contains some germ.	.78	Doubtful (not estimated).	Fairly high. Prevents polyneuritis when added to highly milled corn grits in proportion of 10 gms. to 35 gms.
7	Corn germ.....	Indiana (roller mill).	Germ plus traces of bran.	2.81	High.....	High.
18	Corn meal.....	Maryland (roller mill).	Highly milled...	.29	Low.....	Very low. Produced polyneuritis in 3 weeks.
17	Corn grits.....	North Carolina (roller mill).	do.....	.13	do.....	Do.
9	do.....	Mississippi State Hospital.	do.....	.18	do.....	Do.
10	do.....	Georgia.....	do.....	.17	do.....	Do.
11	do.....	Georgia (U. S. penitentiary).	do.....	.20	do.....	Do.
12	do.....	Mississippi.....	do.....	.17	do.....	Do.
15	do.....	South Carolina.....	do.....	.18	do.....	Do.

TABLE 2.— *P_2O_5 content of various products from same run of corn (Maryland roller mill).*

Sample No.	Nature of product.	Appearance.	P_2O_5 in per cent.
37	Corn.....	Whole.....	0.80
39	"Table meal".....	Considerable bran and germ.....	.81
40	"Table hominy".....	No bran or germ.....	.17
41	"Cream meal".....	do.....	.16
43	Corn flour.....	White; no bran or germ.....	.21
42	Corn germ.....	Largely germ; little bran.....	2.57
44	Corn bran.....	Largely bran.....	.69

TABLE 3.—*Wheat products—content in P_2O_5 and vitamins.*

Sample No.	Nature of product.	Source.	Degree of milling.	P_2O_5 in per cent.	Estimated vitamine content (from feeding experiments).	
					Fat-soluble vitamine.	Antineuritic vitamine.
49	Wheat (whole).....			1.12	Considerable...	High.
61	do.....			1.01	do.....	Do.
86	do.....	South Carolina.....		.89	do.....	Do.
85	"Graham" flour from wheat No. 86.	South Carolina (buhr mill).	Undermilled....	.86	do.....	Do.
92	"Graham" flour.....	do.....	do.....	.82	do.....	Do.
84	Wheat bran.....	do.....	Undermilled; white material adhering to cellulose.	2.12	Probably deficient (not estimated).	Do.
97	"Whole-wheat" flour.....	Missouri (roller mill).	Undermilled; contains some germ and bran.	.62	Considerable...	Do.
98	"Red dog" feed.....	Washington, D. C. (roller mill).	Undermilled....	.92	do.....	Do.
103	"White" middlings.....	do.....	do.....	1.65	do.....	Do.
101	"Brown" middlings.....	do.....	do.....	1.88	do.....	Do.
88	Wheat flour from No. 86.	South Carolina (buhr mill).	Highly milled...	.26	Very deficient..	Deficient.
91	"Patent" flour.....	Virginia.....	do.....	.20	do.....	Very deficient.
94	do.....	Michigan.....	do.....	.21	do.....	Do.
95	do.....	Maryland.....	do.....	.25	do.....	Do.
93	do.....	South Carolina.....	do.....	.22	do.....	Do.

Diagram of flour milling process and distribution of phosphorus.

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